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THE CONCEPT OF FAMILIARITY AND PEST RESISTANT PLANTS¹

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INTRODUCTION

Meetings such as this workshop provide an all too rare opportunity for scientists from different disciplines to share their perspectives on a topic of common interest. In this case we examine the use of pest resistant plants in managed ecosystems. USDA-APHIS has a clear interest in this subject because it is involved in regulating transgenic plants, many of which have been engineered with some sort of pest resistance, within its broad authority to protect plants under the Federal Plant Pest Act and the Plant Quarantine Act. Since 1992, when APHIS received its first request to determine non-regulated status for a transgenic crop, the agency has approved 43 petitions for non-regulated status; 16 of those are for crops with engineered pest resistance. The agency authorizes controlled field testing of transgenic plants in which test plants are isolated from other plants that might be affected. APHIS grants nonregulated status once it determines that the transgenic plant does not present a plant pest risk. In the regulations, the concept of plant pest risk is associated with direct or indirect injury or damage to plants or plant products.

How does APHIS decide if a transgenic plant poses a plant pest risk? As part of its assessment, the agency asks two questions: 1) What is known about the properties of the plant and the environment into which it will be introduced? and 2) What are the probable effects of the plant on the environment?

THE CONCEPT OF FAMILIARITY

Familiarity has consistently been a prominent criterion for evaluating the risks associated with transgenic organisms. The concept of familiarity was presented 10 years ago in the document entitled "Field Testing Genetically Modified Organisms: Framework for Decisions," which was produced by a panel of experts selected by the National Research Council and published by the National Academy of Sciences (NAS). That 1989 NAS report considered how to evaluate the relative safety of testing transgenic plants in the field. The panel summarized some critical observations and principles that were relevant for field testing. APHIS has used these conclusions in the process of assessing transgenic plants, on a case-by-case basis.

APHIS assesses risk by considering what is known about the following factors: the biology of the crop, the introduced trait, the receiving environment, and the interaction between these. The biology of the crop includes, for example, the mating system, mode of pollination, and compatibility with wild relatives. Aspects of the introduced trait to consider include the source of the resistance and how it was introduced. In the case of pest resistance, consideration of the introduced trait also includes the pests to which resistance is conferred. Examples of points to consider about the receiving environment are the presence of sexually compatible wild relatives, pest populations, and the cultivation practices for that crop. Knowledge of and experience with any and all of these factors provide familiarity, which plays an important role in assessments. This concept of familiarity allows the decision-makers to draw upon past experience with introduction

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of plants into the environment, and to compare genetically engineered plants to their non-engineered counterparts.

GENETICALLY ENGINEERED VERSUS CLASSICALLY BRED CROPS

One conclusion in the NAS report is that crops modified by genetic engineering should pose risks that are no different from those of crops modified by classical genetic methods (including bridging crosses, wide crosses, mutagenesis, etc.) for *similar* traits and grown in *similar* environments. Similar traits means traits that produce similar phenotypes in an engineered or a traditionally bred crop, for example: resistance to similar insects in an engineered or a traditionally bred crop; and resistance to similar viruses in an engineered or a traditionally bred crop. A similar environment means an environment similar to one where the plant has always been grown. Generally, plants engineered for pest resistance will be grown in the same places that their non-engineered counterparts have always been grown. One important point of this first conclusion is that it is more important to evaluate the phenotype produced, rather than the process/techniques that were used to produce it. In the context of this workshop, this is a very important point, because what needs to be addressed and focused on are the effects of any pest resistance genes, traditionally bred or engineered, in managed ecosystems.

A second important conclusion made by the panel is that plants modified by classical breeding techniques have a history of safe use. This is not to say that traditional practices pose zero risk, but that the level of risk has been acceptable and manageable. Familiarity does not necessarily mean safe, but that enough is known about the plant to determine the level of safety.

These points are generally agreed upon by scientists who have been concerned with the issue, as in the frequently cited paper by Tiedje *et al.* (1989). In that comprehensive overview of engineered organisms the authors state that “transgenic organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them . . .” and “Long

term experience derived from traditional breeding provides useful information for the evaluation of genetic alterations similar to those that might have been produced by traditional means, and such alterations are likely to pose few ecological problems.”

In many cases, plants developed through genetic engineering and traditional breeding are similar. Consider how a new variety is developed. Traits are initially introduced through genetic engineering or through traditional techniques involving crossing a standard or elite variety with a particular relative that has a desirable trait, such as disease resistance. After a promising new variety has been identified, whether in a greenhouse or a laboratory, it is typically tested in the field for several seasons to see how it performs in a variety of agricultural settings. Once in the field, it may also be backcrossed a number of times to restore the desired genetic background. Regardless of how the trait was initially introduced, the subsequent development follows a well established and formal process.

The information gathered in these steps is extensive. A great number of characteristics are considered in detail during the process of developing a new variety because the developer is keenly interested in being certain that the new variety behaves just like other successful varieties of the crop in as many agronomically significant ways as possible. As part of a petition seeking nonregulated status, APHIS requires applicants to report any differences that are observed between the transgenic lines and the parental organism during this variety development process. So aside from the desired phenotypic change, engineered plants are usually similar to their non-engineered parents, and that allows the agency to assess them based on previous experience with the biology of the crop and its environment and what is known about the introduced trait.

FAMILIAR TRAITS

What kind of traits are we familiar with? Familiarity varies from case to case. Consider one example. Table 1 shows all of the pests in melon for which traditional sources of resistance have been identified and can be used by breeders

(Pitrat 1994). This is part of what forms our basis for familiarity with pest resistance in melon. The only transgenic melons that have been approved by APHIS for field testing have similar non-transgenic phenotypes, which are shown in italics in Table 1. Generally, many traits for pest resistance available from traditional breeding can be used as a base for our familiarity with genetically engineered traits.

Consider, as another example, the transgenic pest resistant plants that APHIS has deregulated or that are pending deregulation. For some of these

plants, there are comparable pest resistant cultivars obtained by traditional breeding (Table 2). Resistance genes found in traditional breeding sources are not the same as those introduced by genetic engineering, but they confer similar phenotypes. In other deregulated pest resistant crops the resistance traits are found in the gene pool, but are not necessarily found in commercial lines. Although there is less experience with these traits that are not found in commercial lines, there is some familiarity with these traits based on reports where these traits have been found in relatives of these crops.

Table 1. Pests for which traditional sources of pest resistance/tolerance exist in *Cucumis melo* (melon).
(Those with comparable field-tested transgenic resistance are shown in italics.)

Aphid	Downy mildew	Pickle worm
Anthraxnose	<i>Erwinia tracheiphila</i>	Powdery mildew
Cucumber scab	Fruit fly	PRSV
CMV	<i>Fusarium oxysporum</i>	<i>Pseudomonas lachrymans</i>
<i>Colletotrichum lagenarium</i>	Gummy stem blight	Root knot nematode
<i>Corynespora melonis</i>	Hypocotyl rot	Spider mites
<i>Corynespora cassiicola</i>	Leaf blight	SqMV
Cucumber beetle	Leaf miner	WMV2
CGMMV	MNSV	ZYMV
Diabrotica		

Table 2. Deregulated transgenic pest resistant phenotypes and genetic resources available for traditional breeding.

<u>Transgenic Plant</u>	<u>Conventional Source of Similar Phenotype</u>	<u>Reference</u>
Lepidopteran resistant corn	Resistant commercial hybrids available	Barry and Darrah 1997
PLRV resistant potato	Resistant cultivars available	Swiezynski 1994
PVY resistant potato	Resistant cultivars available	Khurana and Garg 1998
Coleopteran resistant potato	15 resistant accessions in the genus <i>Solanum</i> L., subgenus Potato, section petota	GRIN 1994
ZYMV, WMV2 resistant squash	Resistant cultivar available	Sold by Harris Moran
CMV resistant squash	Resistant cultivar available	Quemada, pers.comm.
Lepidopteran resistant cotton	Gossypol, Factor X in <i>Gossypium</i> ssp.	Dilday and Shaver 1976; Perceval, pers. comm.
PRSV resistant papaya	Tolerance genes identified	Gonsalves, pers. comm.
Lepidopteran resistant tomato	Resistance in <i>Lycopersicon</i> ssp., particularly <i>L. hirsutum</i>	Stevens and Rick 1986

POTENTIAL FOR PEST RESISTANCE GENES TO ENHANCE WEED PROBLEMS

One of the main concerns with the ecological effects of transgenic plants is that the engineered genes will escape to their wild or weedy relatives and enhance the recipients' weediness in agriculture ecosystems or their invasiveness in natural communities. Is this a probable effect in the case of pest resistance traits?

There are many well-studied examples of hybridization and introgression between domesticated plants and their wild relatives. Many of these involve hybridizations that have been implicated in weed evolution. One of the best examples is Johnsongrass (*Sorghum halepense*), one of the world's most noxious weeds, which arose from the hybridization of cultivated sorghum (*Sorghum bicolor*) and the wild *Sorghum propinquum*. Some of the ecologically important traits thought to have been acquired from the crop include earlier flowering, greater seed production, larger individual seed weight, and earlier emergence (NAS 1989), traits that are often associated with weediness. But there is no evidence that any pest resistance genes from cultivated sorghum have enhanced the weediness of Johnsongrass. In fact, APHIS is not aware of any evidence that weeds have benefited from the acquisition of crop pest resistance genes. Clearly, genes, including pest resistance genes, flow from crops to their sexually compatible wild relatives. The lack of evidence for beneficial effects on weeds may be due either to a lack of effect, or because not enough time and effort have been spent looking for effects.

One thing to consider is that in order for pest resistance to have a noticeable effect in natural populations, the pest itself should have a significant effect on the natural populations. All of the deregulated pest resistant crops have compatible wild relatives somewhere in the world. There is no evidence, however, to indicate that the pests these deregulated crops are engineered to resist have an ecologically significant role in limiting populations of the wild relatives. Is that because no one has looked? Obviously, there are examples of plant pests that do have significant effects on natural populations

of plants. The devastating effects of gypsy moths on forest trees are a striking example of this. Other examples are chestnut blight and Dutch elm disease, both caused by fungal plant pathogens that were introduced into North America during the past century. Clearly, resistance to these pests could have had a significant effect. However, these examples may not reflect the same sort of potential interactions exhibited by some pest-crop-wild relative complexes. In the examples above, the pests are all introduced or exotic species and the hosts are long-lived species that have not co-evolved with them. In contrast, most crop species have co-evolved with their pests, including repeated, often annual, selections mediated by humans.

EVALUATING THE RISK

How should a regulatory agency assess whether genes for resistance to crop pests, traditionally bred or engineered, will confer an advantage on a wild relative that may cross with the crop? This issue needs to be considered on a crop-by-crop and a trait-by-trait basis. To improve the effectiveness of using the concept of familiarity in assessing ecological consequences of pest resistance, some important questions need to be addressed for individual crops.

- ◆ Are there examples of traditionally bred or naturally occurring crop pest resistance genes that confer or enhance weediness? APHIS does not know any examples of a pest resistance gene that has enhanced weediness, but this needs to be addressed in individual crops, and for individual pests or types of pests.
- ◆ Are there examples of pests that limit natural populations of wild relatives of crops where the acquisition of resistance would clearly make a difference? For crops in which pest resistance is being engineered (i.e., against *Rhizoctonia* in the grasses, fungal diseases in strawberries, viruses in the cucurbits, etc.) are there examples where the pests do have a significant effect on the natural populations? Hopefully we will be able to identify other questions over the course of this workshop.

CONCLUSIONS

Familiarity can always be increased as a result of a trial or experiment, and the increased familiarity can then form a basis for future assessments. The Biotechnology Risk Assessment Research Grants Program, administered by the Cooperative State Research, Education, and Extension Service (CSREES) and the Agricultural Research Service (ARS) of the USDA, supports research that will assist Federal regulatory agencies in making science-based decisions about introducing genetically modified organisms into the environment. Proposals should be designed to identify risks, quantify the likelihood of these risks, and quantify their probable effects. Ideally, these grants support projects designed to bring together scientists from many relevant disciplines. Plant breeders, plant pathologists, entomologists, biochemists, molecular biologists, and ecologists should pool their expertise to investigate questions that will increase familiarity with specific issues related to risk assessment.

Returning to the concept of familiarity, two documents referenced in this presentation, Tiedje *et al.* (1989) and the NAS (1989) report, were published ten years ago and were written from a broad perspective on genetic engineering. Reasoning from such broad premises for all organisms and their potential uses can sometimes yield statements that are too general and not always useful. In order to generate useful discussion, it is necessary to identify and focus on specific issues that are components of risk. This workshop on the ecological effects of pest resistance genes in managed ecosystems presents an opportunity to do just that. APHIS recognizes the importance of observational information from individuals who are the true experts on the biology of a particular crop or its pests and does not hesitate to request additional information from those experts when questions arise. APHIS strives to keep its reviews science-based, and it cannot be emphasized strongly enough how

important it is to focus on identified risks supported by facts. Speculation without facts may be valuable, but it is not risk assessment.

The objectives of this workshop are 1) to review existing evidence that the introduction of pest resistance into a crop species has affected the establishment, persistence, and spread of the crop or of species related to the crop; and 2) to identify gaps in the information concerning the ecological effects of pest resistance genes, and recommend strategies to address those. These objectives call us to improve upon that with which we are already familiar regarding pest resistance in crop species.

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